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AUTHOR(S):

Sasai, Tokio; Kubo, Katsuhiko; Kakei, Maroru;
Shinohara, Hideyuki

CITATION:

Sasai, Tokio ...[et al]. Effect of Hyaluronidase on the Blood Mucoprotein in Patients with Carcinomas. Bulletin of the Institute for Chemical Research, Kyoto University 1959, 37(3): 207-212

ISSUE DATE:

1959-09-15

URL:

<http://hdl.handle.net/2433/75702>

RIGHT:

Effect of Hyaluronidase on the Blood Mucoprotein in Patients with Carcinomas

Tokio SASAI, Katsuhiko KUBO, Mamoru KAKEI and Hideyuki SHINOHARA*

(Hayaishi Laboratory)

Received February 16, 1959

It has been reported that there is intimate relationship between hyaluronidase and serum mucoprotein.

In this paper, specimens of serum were examined in 52 human individuals for mucoprotein contents and their changes during the incubation with hyaluronidase or with distilled water.

Serum mucoprotein contents were changed considerably as the result of incubation. In the case of carcinomatous patients, particularly those with carcinoma of stomach, however, serum mucoprotein value was far less susceptible to incubation with hyaluronidase or with water, than the non-carcinomatous patients.

I. INTRODUCTION

The hyaluronidase (abbreviated as H.D. hereafter) was discovered by Meyer in 1936¹⁾ in the autolyzate from type II pneumococcus. Later this came to be identified with the spreading factor²⁾ and has received increasing attention of clinicians. Its enzymatic actions or the serum principles acting against it, namely the non-specific hyaluronidase inhibitor (N.H.I.)^{3) 4) 5)} have been actively investigated.

It is now known that to this complex H.D.-N.H.I. system the serum mucoprotein (abbreviated Mp hereafter) stands an intimate relation. The authors have carried out a clinical study of the enzymatic influences of H.D. on serum Mp.

II. MATERIAL AND METHOD

1) **Material.** Specimens of serum were obtained from a total of 52 patients. They were 13 cases of carcinoma of stomach, 2 cases of carcinoma of the liver, 5 cases of leukemia, 6 cases of liver cirrhosis, 7 cases of inflammatory diseases (cholecystitis, pleurisy *etc.*), 19 cases of other diseases. For this study fresh serum, collected during fasting, was used.

2) **Method.** Each specimen of serum, 0.5 m.l., was examined for Mp contents in three parallel experiments as follows:

A) a portion was examined untreated for the native Mp value.

B) a portion was mixed with 10 units H.D. and incubated for 60 minutes

* 笹井外喜雄, 久保 勝彦, 笥 守, 篠原 秀幸

at 37°C.

C) a portion was used as control being incubated, only together with distilled water, for 60 minutes at 37°C.

Serum Mp contents were determined polarographically after treating the serum with 20% sulfosalicylic acid and collecting the filtrate (for detail see Egawa)⁶⁾. The preparation of H.D. used was "Sprase" manufactured by Mochida Pharmaceutical Company. Solution at the concentration of 10 units per ml was made exactly on the day of the experiments. For the experiment 10 units of H.D. were added to the serum. According to Büchner⁷⁾ the amount of H.D. to be added to the serum to overcome the N.H.I. activity of the serum is 20000 units per 6 liter blood or 3.3 units per ml serum.

We took polarographic activities of H.D. itself into consideration and considered 10 units H.D. per 0.5 ml serum to be the effective minimum dosis. H.D. exerts its activity over the range of pH 4~8, optimally at pH 5. Because the serum protein should be denaturated, we avoided acidic medium by preparing aqueous solution of H.D..

III. RESULTS

1) Native Mucomprotein Value

Among the 52 cases studied 31 cases showed abnormally high values (40mm. or above) of the serum Mp wave. They consisted of the whole group of carcinoma of the stomach (13 cases), 1 case of carcinoma of the liver and 17 cases of non-carcinomatous diseases (inflammatory diseases, leukemia and others). In contrast, five of the six cases of cirrhosis of the liver showed abnormally low value (below 15 mm). These findings are in good agreement with previous reports of many investigators.

2) Effects of Incubation with H.D. and with Distilled Water

a) **Effect of incubation with H.D.** Among the individuals with increased native Mp. values, the carcinomatous group was far less susceptible to H.D. action than noncarcinomatous group. Table 1 indicates that 10 out of 14 carcinomatous patients showed such small changes as below 5 mm, most case toward decrease, while such small changes occurred only in two of the 17 noncarcinomatous patients in which 2 cases of leukemia showed a marked increase. Res-

Table 1. Effect of incubation with H.D.

Effect of incubation with H.D.	Native Mp. Value			
	Cases of high value		Cases of low value	Cases of normal value
	Cancer of stomach, liver	Non-carcino- matous patients		
Small change below 5 mm.	10	2	3	4
Marked change over 5 mm.	4	15	2	12
Total	14	17	5	16

tricting our observation to those with higher native Mp value than 50 mm, then it become that 4 out of the 5 cases with carcinoma of the stomach showed smaller changes than 5 mm, whereas all of the 7 non-carcinomatous patients showed larger changes than 5 mm.

b) **Effect of incubation at 37°C with distilled water only.** As Table 2 indicates changes observed were not uniform in magnitude, its tendency was rather toward decrease. With regards to the carcinoma of stomach 2 out of 8 showed larger changes than 5 mm, while in control groups this was 5 out of 9.

Table 2. Effect of incubation with distilled water at 37°C, 60 min.

Effect of incubation	Native Mp. Value			
	Cases of high value		Cases of low value	Cases of normal value
	Cancer of Stomach, liver	Non-carcinomatous patients		
Small change below 5 mm.	7	4	2	6
Marked change over 5 mm.	4*	5	1	6
Total	11	9	3	12

* 2 out of 4 are the patients with liver carcinoma.

c) **Comparison between the incubation with H.D. and the incubation with water.** The difference between the two types of incubation (one with H.D. the other without) is apparently due to the presence of H.D. in the medium. The changes were smaller in carcinomatous group than in control group (Table 3).

Table 3. Comparison between the incubation with H.D. and incubation with water.

Effect of H.D. itself.	Mp value incubated with water			
	Cases of high value		Cases of low value	Cases of normal value
	Cancer of stomach, liver	Non-carcinomatous patients		
Small change below 5 mm.	8	2	3	5
Marked change over 5 mm.	3	7	0	7
Total	11	9	3	12

3) Variations of Serum Mp Value

In this chapter, variation of serum Mp after incubation was specially analysed from the point of view whether it is high or low against native Mp value.

In Fig. 1 native Mp value was ranged on the OA line, and effect of treat-

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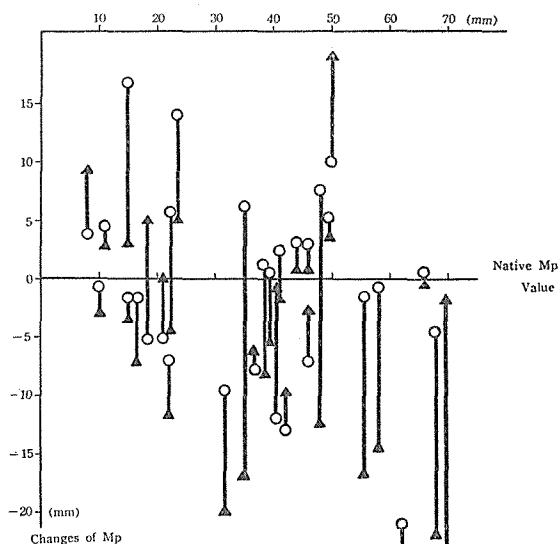


Fig. 1. Variations of Mp value after incubation with H.D. (○) and with distilled water (▲). The projection on the original line indicates the native value of Mp.

ment with H.D. (signed with ○) or distilled water (signed with ▲) was plotted on the vertical direction.

Apparently, it is found that there is an almost parallel relationship between native Mp value and deviation after incubated process.

Moreover, incubation with distilled water only at 37°C was resulted in decreasing tendency of Mp value (decrease, 18 ; increase, 7).

On the contrary H.D. inhibits decreasing tendency of Mp value and promotes the increasing one. Generally speaking, overcoming the influence of incubation, H.D. leads to the increase of Mp.

IV. CONSIDERATION

The authors have thus studied the serum Mp contents under three different conditions, A) untreated, B) treated with addition of H.D. at 37°C, C) with addition of water at 37°C. It was found that the incubation with H.D. or with distilled water for 60 min. at 37°C produces a considerable change in the serum Mp value. When left standing for 60 min. at room temperature, with distilled water no change occurred in serum Mp value. Thus it became evident that the process of incubation at 37°C or the addition of H.D., either independently or as a joint effect, produce changes in Mp value. Interestingly enough the serum of carcinomatous patients was less susceptible to these effects than was the serum from the patients with inflammatory diseases.

In healthy individuals the serum Mp value is maintained within a narrow physiological variation⁸⁾ but considerable changes in serum Mp value are known to occur in association with diseased states⁹⁻¹¹⁾. In the latter circumstances

many electrophoretic¹³⁾ and polarographic¹⁴⁾ changes are known to occur as well as changes in constituents¹²⁾ and origin of mucoprotein¹⁵⁾. These studies are based on *in vivo* experiments and make contrast to our experiments where physico-chemical changes are induced by incubation *in vitro* in test tubes.

A close relation between H.D. and Mp has been discussed¹⁶⁾. That the incubation of serum at 37°C produces changes in serum Mp value suggests active involvement of the already existing serum H.D.-N.H.I. system.

Discovery of N.H.I. or inhibitory action to H.D. dates back to 1932 when Duran-Raynals¹⁷⁾ found the effect of rabbit immune serum to neutralize the infection-promoting action of the testicular extract. As to the mechanism of inhibitions two ways of thinking have appeared: 1) the antibody theory¹⁸⁾ derived from immunological line of investigation and 2) non-specific enzyme theory¹⁹⁾ maintained by Haas with evidence of antivasin I³⁾ (this view was later modified, emphasis being placed on the inhibitory principles rather than on enzymes)⁴⁾. At present analysis of serum N.H.I. is drawing attention. There is a theory that N.H.I. plays a part in the defence mechanism of inflammation⁵⁾. Owing to the presence of N.H.I. there is just trace amount of H.D. in healthy man's blood. Otani²⁰⁾ injected 10000 VRU. of H.D. intravenously in rabbit and found steep decline of its activity within one hour. In view of this study the H.D. transiently entering into the circulating blood, such as occurring presumably in patients with inflammatory or rheumatic diseases, may be counteracted by already increased amount of N.H.I. which immediately inactivated it. By contrast the authors tried to disclose the dynamic conditions of blood H.D.-N.H.I. system at the moment of blood-sampling through the estimation of Mp employing *in vitro* method.

Our study showed that changes in Mp during incubation, with or without H. D. differed from case to case. This suggests that there may be some other mechanism involved besides that of H.D.-N.H.I. system. At this point one may focus his attention on the problem of serum large sized protein. From his adding-recovery experiment, Winzler found the phenomenon of "coprecipitation"; the amount of free type Mp, recovered in the sulfosalicylic acid filtrate, is about 70% of the total Mp, and this figure may vary in different conditions of serum protein and others. The observed inconsistency in the authors' data may be due to different grade of admixture between the free and combined types of Mp. Presumably the degree of admixture is not constant from time to time, being different levels of steady state.

The Mp value being influenced by various factors of internal milieu, the observed changes in Mp value can hardly be ascribed to a single factor; important factors are: the predominant causes of Mp change, the influence of H.D.-N.H.I. system, the effect of adding H.D.. At any rate the fact remains to be of interest that the serum Mp was less susceptible to H.D. in the case of carcinomatous patients (particularly in carcinoma of stomach) as compared with the patients with diseases characterized by interstitial reactions such as inflammation or rheumatism.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to Prof. Gyoichi Wakisaka for his constant interest toward this work.

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